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Oxidant and antioxidant status in patients undergoing percutaneous coronary interventions

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ABSTRACT

Background: Coronary artery disease (CAD) is the major disease leading to cause mortality and morbidity. The imbalance between the generation of reactive oxygen species (ROS) and the intrinsic antioxidant defense system leading to the oxidative stress, has been implicated in the pathogenesis of the cardiovascular disease. The formed free radicals and subsequent lipid peroxidation may be responsible for myocardial damage in patients undergoing percutaneous coronary interventions. Therefore, the understanding of the pathophysiological role of ROS generated during or after coronary interventions is essential to improve the success rate of these procedures. **Aims and Objective:** The aim of this study was to evaluate the time course changes of oxidant and antioxidant status, in patients undergoing percutaneous coronary interventions (PCI). **Materials and Methods:** The study included 120 consecutive patients (117 males, 3 females; mean age 58.4 years) who underwent elective PCI. Coronary angiography and coronary angioplasty were performed according to the standard protocols. Blood samples were taken just before (0hrs) and at 4 hrs and 24 hrs after coronary interventions to determine the oxidative status i.e. plasma malondialdehyde (MDA) and for antioxidative status, erythrocyte glutathione peroxidase (GPx), erythrocyte glutathione (GSH) and plasma ferric reducing ability of plasma (FRAP). **Results:** There was significant increase in MDA levels (1.87 ± 0.34, 1.90 ± 0.46, $p = 0.000$), at 4hrs and 24hrs after coronary interventions when compared to baseline levels. After coronary interventions, the GPx activity (12.96 ± 8.37, 12.3 ± 7.76, $p = 0.000$) and FRAP levels (0.73 ± 0.21, 0.70 ± 0.23, $p = 0.001$ respectively) were found to be increased significantly at 4hrs and 24hrs. However, the glutathione levels (2.40 ± 0.57, 2.47 ± 0.53, $p = 0.040$) were decreased at 4hrs and 24hrs after coronary interventions. **Conclusion:** Our study demonstrates that presence of oxidative stress in coronary interventions. The increase in antioxidants in the present study may be due to paradoxical increase in oxidant levels in patients undergoing coronary interventions.

Key words: Coronary artery disease, Percutaneous coronary interventions, Angioplasty, malondialdehyde, Oxidative stress, Total antioxidant capacity

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INTRODUCTION

The cardiovascular disease has been increasing worldwide and is the leading cause of death in new millennium.[1] Coronary artery disease (CAD) is the primary disease of cardiovascular diseases consists of multifactorial etiologies, which include various biological, environmental, behavioral and socio cultural factors.[2] In addition to these risk factors, several experimental and clinical studies have shown that oxidative stress mediated by reactive oxygen species (ROS) is involved in the pathogenesis of CAD.[3] A percutaneous coronary intervention (PCI) is the non-surgical procedure, most commonly used technique for mechanical revascularization of occluded coronary arteries.

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Ischemia followed by reperfusion involves number of events leading to the over production of oxidants, which can overcome the antioxidant production.[4] It was reported that oxidative stress due to the disturbance in balance between the production of ROS and antioxidant defence plays a vital role in the pathogenesis of atherosclerosis. This may lead to an increase in oxidants and decrease in antioxidants may impair the oxidative/antioxidative equilibrium towards the oxidative status.[5] Increased oxidative status may initiate lipid peroxidation in cell membranes, endothelial dysfunction, or cause DNA fragmentation, vascular smooth muscle cells growth and severe myocardial cell damage.[6]

Studies investigating oxidative stress during PCI yielded conflicting results.[7,8] So far, there are no studies on the combined oxidative and antioxidative status in the coronary interventional procedures. In the current study, we investigated the alterations in oxidative and antioxidative status during coronary angioplasty and coronary angiography using oxidative stress parameter MDA, antioxidant defence system (glutathione peroxidase, glutathione) and total antioxidant capacity (ferric reducing ability of plasma). Our ultimate goal was to evaluate the time course changes in MDA levels and antioxidant defence to understand the ongoing oxidative process in the interventional procedures.

MATERIAL AND METHODS

We performed a single centre, prospective cohort study. A total of 120 patients who undergoing elective percutaneous coronary interventions in the Cardiology Division of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, during January to October 2012 were enrolled. The study was approved by the institutional ethical committee and informed consent was given by all patients. Patients with baseline serum creatinine of ≤1.2 mg/dL for males and ≤1.1 mg/dL for females are included. Exclusion criteria for patients included, patients with pre-existing renal disease, hypotension, hyperthyroidism, hypothyroidism, those on glucocorticoid therapy, cardiogenic shock, patients with normal coronary arteries and patients not willing to participate in the study. Baseline data was obtained from all patients including height, weight, medical history, drug intake, history of diabetes mellitus, hypertension, smoking and alcohol consumption. Body mass index (BMI) (kg/m^2) was calculated. Coronary angiography and angioplasty was performed using the standard procedures.

Blood sampling

Venous blood samples were obtained into heparin-treated tubes just before the PCI procedure i.e. (0hr), 4hrs and 24hrs after the PCI procedure. The blood specimens were centrifuged immediately at 3000rpm for 15min and plasma was separated. The erythrocytes were separated from the heparinized sample, washed thrice with normal saline and equal volume (1:1) of ice cold distilled water was added to lyse the erythrocytes to obtain the hemolysate. The plasma and hemolysate samples were aliquoted and stored in separate vials at -80°C in deep freezer (Thermo Fischer Scientific, USA) until analysis. Plasma was used for the estimation of malondialdehyde (MDA) and ferric reducing ability (FRAP) and hemolysate for the estimation of glutathione peroxidase (GPx) and reduced glutathione (GSH). The measurement of MDA, which is an important marker of oxidative stress, is achieved by spectrophotometric analysis of thiobarbituric acid reactive products (TBARS).[9] Gpx activity was estimated by the method described by Wendel.[10] GSH in blood was estimated by 5, 5-dithiobis 2-nitro benzoic acid (DTNB), as described by Beutler.[11] Total antioxidant capacity (TAC) was determined by ferric acid reducing ability of plasma (FRAP) method.[12] The estimations were performed using Lambda25 UV-visual double beam spectrophotometer Perkin Elmer, Singapore (Spectro photometric analysis).

Statistical analysis

SPSS version 11.5 (SPSS Inc, Chicago, IL, USA) and MedCalc version 12.2.1 (Broekstraat, Mariakerke, Belgium) were used for analyses. Continuous variables were tested for normal distribution with Kolmogorov-Smirnov-test and expressed as mean ± SD. Categorical variables are expressed as frequency (number [%]). The sources of variation at different time points were assessed by analysis of variance (ANOVA), followed by Post Hoc test with Bonferroni's multiple comparisons. A p value <0.05 was considered statistically significant.

RESULTS

No complications were encountered during coronary angiography and coronary angioplasty. Successful reperfusion was obtained in all angioplasty procedures.

Baseline and clinical characteristics of the patient population are summarized in Table 1.

Ninety-eight percent out of 120 patients were males and the mean age was (51.3±8.9). Smokers constituted 61% of the patient population and hypertension was detected in 26% of patients. The patients total cholesterol, high density lipoprotein and low density lipoprotein levels were measured in the patient population. Beta-blockers and ACE inhibitors were used in 13% and 12% respectively in patients.

The time course changes of oxidants and antioxidants at 0hrs, 4hrs and 24hrs are shown in Table 2. Indicator of oxidative stress i.e. MDA levels increased in higher significance at 4hrs and 24hrs compared to 0hrs i.e. before the interventional procedure (1.87±0.34; p=0.000, 1.90±0.46; p=0.002, respectively). The GPx activity increased at 4hrs(12.96±8.37; p=0.000) and 24hrs (12.3 ± 7.76; p=0.000) compared to the 0hrs. Compared to 0hrs, GSH levels were found to be significantly lower at 4hrs (2.40±0.57; p=0.036) whereas no statistical significance was noted at 24hrs. The measure of TAC as FRAP, showed a significant increase at 4hrs (0.73±0.21; p=0.000) and 24hrs (0.70±0.23; p=0.015) compared to 0hrs in the patient population.

Table 3 shows the significant p values at different time points of oxidant and antioxidant markers.

Table 1: Baseline and clinical characteristics of the patient population

Variables	Patients (n=120)
Age (years)	51.3±8.9
Male, n (%)	117 (97.5)
BMI (kg/m^2)	23.8±2.96
Systolic blood pressure (mm Hg)	118.3±12.7
Diastolic blood pressure (mm Hg)	76.6±7.95
Hypertension, n (%)	31 (25.8)
Smokers, n (%)	73 (60.8)
Cholesterol (mg/dL)	137.7±33.0
Triglycerides (mg/dL)	168.8±104.9
HDL-C (mg/dL)	31.9±7.46
Hemoglobin (g/dL)	12.6±1.58
eGFR by CG equation (mL/min)	89.4±26.0
Congestive heart failure, n (%)	21 (17.5)
LVEF (%)	51.5±10.0
CAG/CAG+PCI, n (%)	49 (40.8)/71 (59.1)
Angiographic characteristic, n (%)	
One vessel	74 (61.6)
Two vessels	29 (24.1)
Multi-vessels	17 (14.1)
Drugs	
ACE inhibitor, n (%)	2 (1.7)
Diuretics, n (%)	12 (10.0)
β- Blockers, n (%)	13 (10.8)
Aspirin, n (%)	108 (90.0)
Statins, n (%)	29 (24.2)

Data presented as mean±SD, or numbers (percentage), SD: Standard deviation, n: Number of patients, BMI: Body mass index, eGFR: Estimated glomerular filtration rate, HDL-C: High density lipoprotein-cholesterol, CG equation: Cockcroft Gault equation, LVEF: Left ventricular ejection fraction, CAG: Coronary angiography, PCI: Percutaneous coronary intervention, ACE inhibitor: Angiotensin-converting enzyme inhibitor

DISCUSSION

Reactive oxygen species are produced in all biological systems leading to oxidative stress plays an important role in the pathophysiology of CAD which poses a serious threat to these patients. Oxidative stress as a result of atherosclerosis has been demonstrated previously and was thought to be the principle mediator of ischemia in CAD. During the process of ischemia, there is over production of free oxygen radicals leading to the impairment of oxidative-antioxidative balance which favors the oxidation.[13] There are several studies, implicating excessive oxidative stress in the pathogenesis of cardiovascular risk and disease.[14,15] Plasma MDA is an accurate index of oxidative stress, which denotes total plasma defense against ROS[4] found to be increased in our study which is the end product of lipid peroxidation. In consistent with the previous literature, we observed a significant increased MDA levels in our patient population[4,16] which may be related to the ischemia-reperfusion mechanism. Percutaneous coronary angioplasty represents a clinical model of transient ischemia-reperfusion which involves the lipid peroxidation of membrane poly unsaturated fatty acids caused by ROS. The complete or partial restoration of oxygenated blood flow leads to the

Table 2: Time course changes in oxidant and antioxidant markers of patient population

Variables	0 hr	4 hr	24 hr	p value
Plasma MDA (µmol/L)	1.73±0.30	1.87±0.34	1.90±0.46	0.000*
Erythrocyte GPx (U/gm Hb)	9.82±6.46	12.96±8.37	12.3±7.76	0.000*
Erythrocyte GSH (mg/dL/gm of Hb)	2.52±0.57	2.40±0.57	2.47±0.53	0.040*
Plasma FRAP (mmol/L)	0.69±0.23	0.73±0. 21	0.70±0.23	0.001*

Data is presented as mean±SD, SD: Standard deviation, MDA: Malondialdehyde, GPx: Glutathione peroxidase, GSH: Reduced glutathione, FRAP: Ferric reducing ability of plasma. *=p<0.05 statistically significant

Table 3: The p values of oxidant and antioxidant markers at different time points

Variables	0 hr vs 4hr	0 hr vs 24 hr	4 hr vs 24 hr
Plasma MDA (µmol/L)	0.000*	0.002$^+$	1.000
Erythrocyte GPx (U/gm Hb)	0.000*	0.000$^+$	0.761
Erythrocyte GSH (mg/dL/gm of Hb)	0.036*	0.168	1.000
Plasma FRAP (mmol/L)	0.000*	0.015$^+$	1.000

MDA: Malondialdehyde, GPx: Glutathione peroxidase, GSH: Reduced glutathione, FRAP: Ferric reducing ability of plasma,*p<0.05 when compared to baseline levels (ohr vs 4hr), $^+$p<0.05 when compared to baseline levels (ohr vs 24hr)

increase in concentration of oxygen during reperfusion impairs the balance favoring the anti-oxidative process. The excess production of oxygen leads to generation of ROS, which may initiate the peroxidation of lipids in cell membranes. Thus, ischemia-reperfusion process causes enhancement of ROS leads to lipid peroxidation and cytotoxic damage, suggesting that ischemic injury is the major factor in pathogenesis of CAD.[17]

In accordance with some previous studies, we found that antioxidant levels did not decrease, furthermore some markers increased significantly.[18] Simic D et al[19] reported that in patients with acute myocardial infarction (MI) after successful reperfusion, a significant increase was noted in GPx activity at the first hr and continued up to the third day. Similarly, in the present study a significant increase was observed in GPx activity 4hrs and 24hrs compared to the baseline levels. The significant increase in GPx in our study represents the first line of defence against oxidative damage and is rapidly depleted. However, a significant decrease was noted in GSH at 4hrs and no change at 24hrs when compared to the baseline levels. The decrease in GSH levels are in agreed with Chiaraa BD et al[20] and Kharb S et al[21]. GSH plays an important role in auto oxidation of free radicals against myocardial injury and depressed GSH levels probably reflect the scavenger role against ischemia and reperfusion injury.

We measured the total antioxidant capacity (TAC) in the present study which combines the concentrations of individual antioxidants and reflects the overall antioxidant capacity known as FRAP which is found to be increased significantly at 4hrs and 24hrs when compared to baseline levels. To the best of our knowledge, no studies have yet studied TAC levels in the coronary interventional studies. The increased TAC levels could paradoxically reflect a high OS evidenced by increased MDA levels in patient group that has stimulated the compensatory up-regulation of antioxidants.

Our results provide further evidence for increased oxidative stress associated with PCI, similar to that of ischemia-reperfusion injury mechanism, and suggest that oxidative stress is the common event occurring during the process of ischemia. However, the time course changes and activities of antioxidant enzymes may be affected by the complication and severity of acute coronary event, and by therapeutic interventions such as reperfusion in our study. Another reason may be the enzyme activity can varies with different timings may lead to increased within-study and between-study variability with the onset of several acute coronary events. The activities of enzymes can be modified by medications i.e. statins and also depends on lifestyle and diet. Therefore, evaluation of antioxidant enzyme activity levels in interventional studies needs to be investigated in future clinical studies for better management of disease. The results of the present study would enable useful information in determining the oxidative and antioxidant defense system in patients undergoing coronary interventions.

CONCLUSION

In conclusion, our data suggest the presence of oxidative stress during coronary interventions. Our results provide further evidence of increased oxidative stress with PCI, and suggest that oxidative stress is the common event occurring during the process of ischemia. The time course changes in antioxidant enzyme levels may be affected by several factors and needs to be studied further in future. We therefore suggest the measurement of antioxidant enzyme levels to be further investigated in large number of studies.

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Limitations of the study

The study includes small number of patients and is a single centre study. The study needs to be further evaluated in multiple centers and also with large cohort of patients. Another limitation is, the patients were taking drugs (beta blockers, angiotensin converting enzyme inhibitors and statins), which can affect the levels of oxidative and antioxidative markers.

REFERENCES

1. Surekha RH, Srikanth BB, Jharna P, Ramachandra RV, Dayasagar RV and Jyothy A. Oxidative stress and total antioxidant status in myocardial infarction. Singapore Med J 2007; 48:137-142.
2. Kannel WB and Larson M. Long-term epidemiologic prediction of coronary disease. The Framingham experience. Cardiology 1993;82:137-152.
3. Young IS and McEneny J. Lipoprotein oxidation and atherosclerosis. Biochem Soc Trans 2001; 29:358-362.
4. Mustafa G, Ali Y, Recep D, Remzi Y, Altan K, Ekrem K, et al. The effect of coronary angioplasty on oxidative and antioxidative status. Arch Turk Soc Cardiol 2007;35:21-27.
5. Lakshmi SV, Padmaja G, Kuppusamy P and Kutala VK. Oxidative stress in cardiovascular disease. Indian J of Biochem Biophys 2009; 46:421-440.
6. Chen LY, Nichols WW, Hendricks J and Mehta JL. Myocardial neutrophil infiltration, lipid peroxidation, and antioxidant activity after coronary artery thrombosis and thrombolysis. Am Heart J

1995; 129:211-218.

7. Blann A, Midgley H, Burrows G, Maxwell S, Utting S, Davies M, et al. Free radicals, antioxidants, and endothelial cell damage after percutaneous transluminal coronary angioplasty. Coron Artery Dis 1993; 4:905-910.
8. Roberts MJ, Young IS, Trouton TG, Trimble ER, Khan MM, Webb SW, et al. Transient release of lipid peroxides after coronary artery balloon angioplasty. Lancet 1990; 336:143-145.
9. Sangeetha P, Das UN, Koratkar R and Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic Biol Med1990; 8:15-19.
10. Wendel A. GPX: In. Jakoby W.B.eds. Methods in Enzymology-77 NY. Academic press 1981:325-333.
11. Beutler E, Duran O and Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Med 1963; 51:882-888.
12. Benzie IF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996; 239:70-76.
13. Belch JJ, Bridges AB, Scott N and Chopra M. Oxygen free radicals and congestive heart failure. Br Heart J 1991; 65:254-258.
14. Martinet W, Knaapen MW, De Meyer GR, Herman AG and Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circulation 2002; 106:927-932.
15. Andreassi MG. Coronary atherosclerosis and somatic mutations: An overview of the contributive factors for oxidative DNA damage. Mutat Res 2003; 543:67-86.
16. Rigattieri S, Buffon A, Ramazzotti V, Mordente A, Crea F, Maseri A, et al. Oxidative stress in ischemia reperfusion injury: Assessment by three independent biochemical markers. Ital Heart J 2000; 1:68-72.
17. Hakan TM, Kalkan ME, Aksakal E, Simsek Z, Bakirci EM and Alp HH. Oxidative and Antioxidative Status after Successful Percutaneous Transluminal Coronary Angioplasty in Acute Myocardial Infarction. J Clin Trial Cardiol 2014; 1:1-4..
18. Perrelli MG, Pagliaro P and Penna C. Ischemia/reperfusion injury and cardio protective mechanisms: role of mitochondria and reactive oxygen species. World J Cardiol 2011; 6:186-200.
19. Simic D, Mimic-Oka J, Pljesa M, Milanovic D, Radojevic S, Ivanovic B et al. Time course of erythrocyte antioxidant activity in patients treated by thrombolysis for acute myocardial infarction. Jpn Heart J 2003; 44: 823-832.
20. De Chiara B, Mafrici A, Campolo J, Famoso G, Sedda V, Parolini M, et al. Low plasma glutathione levels after reperfused acute myocardial infarction are associated with late cardiac events. Coron Artery Dis 2007; 18:77-82.
21. Kharb S. Low blood glutathione levels in acute myocardial infarction. Indian J Med Sci 2003;57: 335-337.

Authors Contribution:
VLAL - Literature search, collected samples and analyzed data, reviewed the literature, manuscript preparation and critical revision of the manuscript; **MMS -** Concept and reviewed the literature, helped in preparing first draft and critical revision of the manuscript; **ARRB -** Statistically analyzed and interpreted the data, conceptualized study, literature search and reviewed the literature; **PVLNSR -** Concept of study, statistically analyzed and interpreted the data and reviewed the literature; **DRS -** allowed for sample collection.

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